EFFECTS OF FUMIGANTS ON THE RESPIRATORY MECHANISMS OF *TENEBRIO MOLITOR* (L.)

LENG HONG TEO
Department of Entomology
University of Alberta, Edmonton

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Fumigation of larvae and adults of Tenebrio molitor *(L.) for five hours with ethylene dichloride at 0.12 gm/1 resulted in an apparent increase in oxygen consumption during treatment. Sorption of the fumigant by the insect tissue caused a decrease in pressure in the manometer which would ordinarily be construed as an increase in oxygen consumption. A 45 minute treatment caused hyperactivity and an increase in ventilation movements in larvae resulting in an increase in oxygen consumption. Paralyzed adults and anaesthetized insects showed no change in oxygen consumption after treatment but high doses of ethylene dichloride depressed oxygen consumption by homogenized tissue. Similar treatment with carbon disulphide caused no significant change of oxygen consumption in adults or larvae, but larvae were paralyzed sooner than by ethylene dichloride. Carbon disulphide depressed oxygen consumption by anaesthetized insects and by homogenized tissue, the effect being greater at higher doses. Sorption of carbon disulphide was faster than that of ethylene dichloride. Both fumigants caused the second and third spiracles of* Tenebrio molitor *adults to open when applied locally to the ventral nerve cord.*

Fumigants are toxic chemicals which enter the bodies of insects in gas form, chiefly through the spiracles, but also through the integument (Bond 1961). The opening and closing of the spiracles is the chief factor controlling the amount of fumigant which enters the body. Since the respiratory rate is closely correlated with these spiracular movements, fumigants affect it. For example, the susceptibility of insects to methyl bromide is closely related with the respiratory rate (Bond 1956) and hydrogen cyanide, which is a respiratory poison acting on the cytochrome system, increases the resistance of some insects to methyl bromide (Bond 1961).

The finding that carbon dioxide can affect the movement of isolated or denervated spiracles (Beckel & Schneiderman 1957, Case 1957, Miller 1960b, Hoyle 1961) through neuromuscular transmission (Hoyle 1960) raises the question as to whether fumigants affect spiracular movement irrespective of their effects on the rate of respiration. Bond (1961) found it difficult to attribute spiracular condition to any particular action of the fumigant or to relate uptake of the fumigant to the spiracular condition. Kitchel & Hoskin (1935) found an irregular response of the spiracles of Hawaiian cockroaches *Nyctobora noctivaga* (Rehn) to nicotine concluding that nicotine deranges the mechanism of spiracle control. Wigglesworth (1941) showed that the spiracles of *Cimex lectularius* were closed most of the time when paralysis caused by pyrethrins was complete.

Shafer (1911, 1915) found that, when a tissue extract of *Passalus cornutus* Fab. was treated with carbon disulphide, oxygen consumption was depressed and oxidase and catalase strongly inhibited. De Meio & Brieger (1949) found that rabbit kidney, liver, and brain tissues treated with 0.01 M carbon disulphide, showed no decrease in oxygen consumption, but carbon disulphide reacts with reduced glutathione (Anonymous

1949), affects oxidase and catalase (Shafer 1915), and inhibits the succinic oxidase enzyme system (McKee *et al.* 1943).

The effect of ethylene dichloride on insect respiration has not been studied. Working on *Musca domestica* L., Winteringham &Hellyer (1954) found that ethylene dichloride vapour induced deep narcosis within 5 minutes, with only a very slight fall in the levels of adenosine triphosphate and arginine phosphate. Exposure for one hour caused considerable depletion of ATP and arginine phosphate but the phosphoglycerate level was unaffected. They concluded that the delayed depletion of adenosine triphosphate by ethylene dichloride indicates that narcotics impede the oxidative synthesis of this material but the immediate narcosis with little depletion of ATP within the first few minutes does not support this view. Furthermore, ethylene dichloride does not react with reduced glutathione (Anonymous 1949). This indirect evidence indicates that ethylene dichloride is not likely to affect the respiration of insects.

MATERIALS AND METHODS

I measured oxygen consumption by *Tenebrio molitor* L. during treatment with fumigants; some of the insects were first treated with fumigants, then their oxygen consumption was determined. The insects were classified as active if they still moved, or paralysed if they were lying on their sides or backs, to assess the part played by activity in the variations in oxygen consumption observed. The larvae were better for this study because they are not so readily paralysed. To eliminate the effect of activity oxygen consumptions by homogenized tissue and by anaesthetized insects were determined after treatment with fumigants. The effect of fumigants on ventilation prior to paralysis was observed. After paralysis, the condition of spiracles is the chief factor affecting the amount of fumigants entering the body and so this was also recorded.

The insects were obtained from a culture reared at 26 C. About twenty-four hours before the test, the insects were put in separate containers without food. Mature larvae of *Tenebrio molitor* L. and adults three to six days after emergence were used.

Oxygen Consumption

Oxygen consumption was measured in a Warburg constant volume respirometer with one insect in each flask. Carbon dioxide was removed by filter paper soaked with 0.1 ml of 10% potassium hydroxide in the center well. The experiments were run at 25 C. Oxygen consumption before fumigation was determined first and then the air in the manometer and flask was replaced by a fumigant-air mixture. The method used was essentially that of Umbreit *et al.* (1964). The manometers were connected together with plastic tubes. The last manometer was connected to a flask containing fumigant-air mixture and the first manometer to a vacuum line with a side arm to a manometer for measuring absolute pressure in the whole system. The rubber tubes which served as reservoirs for Brodie's fluid at the lower ends of the manometers were clipped at the upper ends to prevent the rising of the fluid into the manometers when

the system was under vacuum. The whole system was first evacuated to an absolute pressure of 6 cm mercury or lower and then refilled with fumigant-air mixture. This was repeated three times and then the mixture was continuously flushed through the system for ten minutes. The system was closed and ten minutes allowed for temperature equilibration before oxygen consumption was measured for the balance of the 5 hr fumigation period. For measuring the oxygen consumption after fumigation, the air was replaced by the fumigant-air mixture, the system was closed for 45 minutes, and the mixture was then replaced by air. In each of these tests, two of the tubes served to measure the respiration of control insects. Oxygen consumption over successive 30 minute periods was determined in all tests. Between tests respiratory manometers were disconnected, each flask was aerated, and filter papers moistened with potassium hydroxide were replaced.

To obtain a desired concentration of fumigant, the calculated quantity was injected with a micro-syringe into a 6.7 litre flask evacuated to half atmospheric pressure or lower. The flask was fitted with a rubber bung with 2 holes. One of these carried two glass stopcocks leading to the respirometer and joined by a rubber coupling; the fumigant was injected through the hole in the second stopcock, which was disconnected for this purpose. The second carried a glass tube to the bottom of the flask, coupled to the water supply and controlled by a clamp. The rubber stopper of the flask was covered with aluminum foil to prevent sorption of fumigant by the rubber. Water was run into the flask when fumigant was flushed through the respirometer.

Tissue homogenates were prepared by grinding the tissue with a homogenizer in Krebs-Ringer's phosphate solution buffered at pH 6.7 (Umbreit *et al.* 1964) which is about the middle of the pH range of tissues of *T. molitor* (Roeder 1953). For each test, ten adults or six larvae were used and the homogenate was prepared in 25 ml of buffer solution. The homogenate was filtered through several layers of cheese cloth to eliminate fragments of cuticle. About two-thirds of the tissue homogenate was then transferred to a vial which was put into a copper-screen tube with a diameter of 2.8 cm and height 7.5 cm. Then the homogenate was exposed to a known concentration of fumigant for a period of time, after the method of Richardson & Casanges (1942). The fumigant was injected into a 3.1 litre Erlenmeyer flask as described above. After vapourization was complete and air had been flushed in, the original stopper was replaced quickly with another stopper, from which the copper-screen tube with the vial containing homogenate was suspended. After treatment, the homogenate was placed in the respiratory flasks which were weighed previously and the flasks with the homogenate were weighed again. The oxygen consumption by the homogenate of four treated and three control samples was then determined. The pH of the homogenate was measured before and after treatment with fumigant and also after oxygen consumption was measured and it remained stable in the treated and control samples.

For studying the effects of fumigants on oxygen consumption by insects anaesthetized with chloroform, some adults and larvae were first anaesthetized by putting them into a flask saturated with chloroform and

removing them after they became motionless. They were then treated with ethylene dichloride or carbon disulphide for 0.75 hour and their oxygen consumption over a period of one hour was measured. The control insects were similarly anaesthetized with chloroform before their oxygen consumption was determined. Homogenized larval tissue was treated with chloroform saturated in air for 0.25 hour to see if chloroform affects tissue respiration. Four treated and three control samples were used.

Sorption by Insects

To see whether the insects themselves remove any quantity of fumigants during treatment, the insects were put into a copper-screen tube with cover, exposed to a known concentration of fumigant and weighed at intervals. To minimize water loss from the insects, the relative humidity in the flask was kept high by 5 ml of water run into the flask on the day before the test.

Respiratory Movements

A petri dish with the upper rim greased before covering was used to contain insects while spiracular movements were observed under a binocular microscope. The test insect with wings removed to expose the spiracles was mounted on a piece of molding clay and the fumigant was injected with a micro-syringe onto a small piece of cotton wool in the petri dish to give concentrations of 0.12 and 0.24 gm/l, and allowed to vapourize. Spiracles with associated structures were also dissected from the insects and placed on a small cotton ball soaked with Ringer's solution in a petri dish to which fumigant was introduced. Observations on the effects of fumigants on abdominal ventilation movements were made in the same way.

Observations on the time to paralysis were made by exposing insects to a known concentration of fumigant in an Erlenmeyer flask as with homogenized tissue.

To study the effect of ethylene dichloride and carbon disulphide on the activity of the ventral nerve cord, male *Periplaneta americana* (L.) adults were used. They were decapitated, mounted in a wax dissecting dish, and the body was opened through the dorsum exposing the ventral nerve cord which was then slightly raised and placed on a pair of platinum wire electrodes leading to an oscilloscope.

RESULTS

Oxygen Consumption during Fumigation

Both sexes of *T. molitor* adults showed an initial increase of oxygen consumption one hour after the treatment with ethylene dichloride started at a concentration of 0.12 gm/l (0.0012 M), the increase being more pronounced in the males than in the females (fig. 1). Oxygen consumption reached its peak one hour after the treatment started, and gradually decreased reaching its original level an hour after treatment finished. When the treatment was just started, the beetles showed more activity.

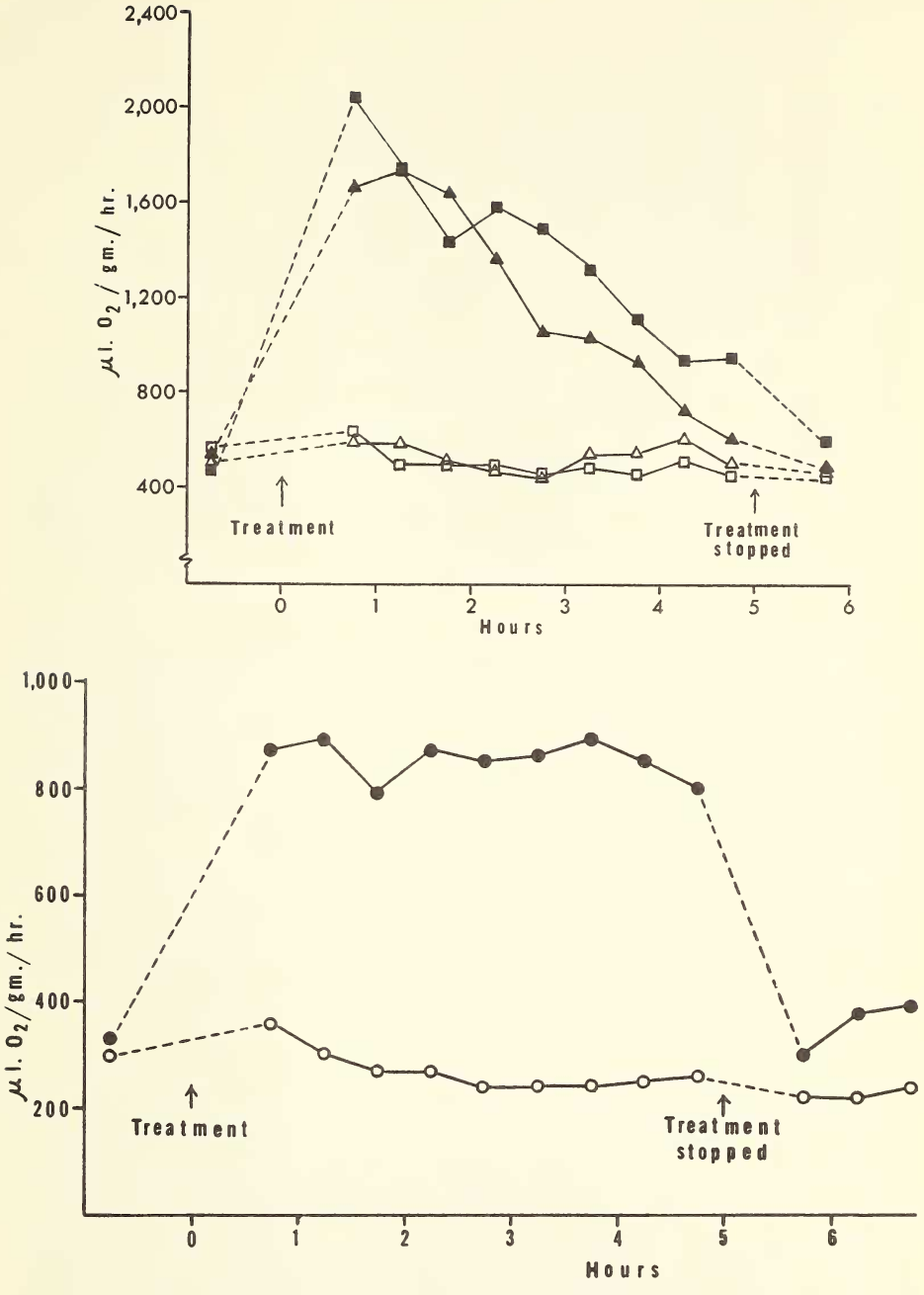

Fig. 1. Mean oxygen consumption by *Tenebrio molitor* before, during and after fumigation with ethylene dichloride (0.12 gm/l.). Upper: adults; lower: larvae. ■ 7 treated males, □ 2 control males; ▲ 7 treated females, △2 control females; ● 7 treated; ○ 2 control insects.

This probably would not account for all of the increase in oxygen consumption because most were paralysed in less than fifteen minutes.

When the larvae were fumigated with the same concentration of ethylene dichloride, there was an increase in oxygen consumption and this was maintained throughout the whole fumigation period (fig. 1). There was a sharp drop towards the original level after the treatment stopped. After fumigation started, hyperactivity occurred for some time before paralysis set in. These restless movements lasted for an hour or longer accounting for part of the increase in oxygen consumption. Part of the pressure change may have been due to sorption of the fumigant by the insects, since there was a sharp drop in apparent oxygen consumption when fumigation stopped.

Fig. 2 shows the results when the adults were fumigated with carbon disulphide at a concentration of 0.12 gm/l (0.0016 M). The males and females showed no marked changes of oxygen consumption in comparison with the controls during and after the treatment. Fig. 2 also shows the oxygen consumption rate during the period when the larvae were fumigated with carbon disulphide at a concentration of 0.12 gm/l. A sudden increase was followed by a sharp decrease and two hours after fumigation started, the rate of oxygen consumption by the treated insects was essentially the same as that by the control.

Oxygen Consumption after Treatment

As shown in fig. 3, there were wide variations among the individuals in oxygen consumption after treatment with ethylene dichloride which did not correlate with the insects' activities. However, when an insect was incapacitated immediately after treatment, there were some spasms, although no increase of oxygen consumption. When hyperactivity occurred for a longer period there was a corresponding higher rate of oxygen consumption.

When the adult females were similarly treated, there was essentially no difference in oxygen consumption between the treated and the control (fig. 4). The insects were all paralysed after the treatment.

Another lot of larvae was similarly treated with carbon disulphide (fig. 4). There was no hyperactivity or increase of oxygen consumption after the treatment. Similar results were obtained with adult females (fig. 4).

Effects on Homogenized Tissue

Ethylene dichloride at 0.12 gm/l x 1 hr greatly increased the oxygen consumption by most of the *Tenebrio molitor* larvae through its irritating effect, but this dosage depressed slightly the consumption by larval tissue homogenates. A higher dosage was required to depress the oxygen consumption by homogenized tissue of adult males and females (table 1). Carbon disulphide at 0.12 gm/l x 1 hr depressed the oxygen consumption by homogenized tissue of larvae and of adults of both sexes, the effect being greater in adults.

Effects on Anaesthetized Insects

Carbon disulphide depressed the oxygen consumption by both the

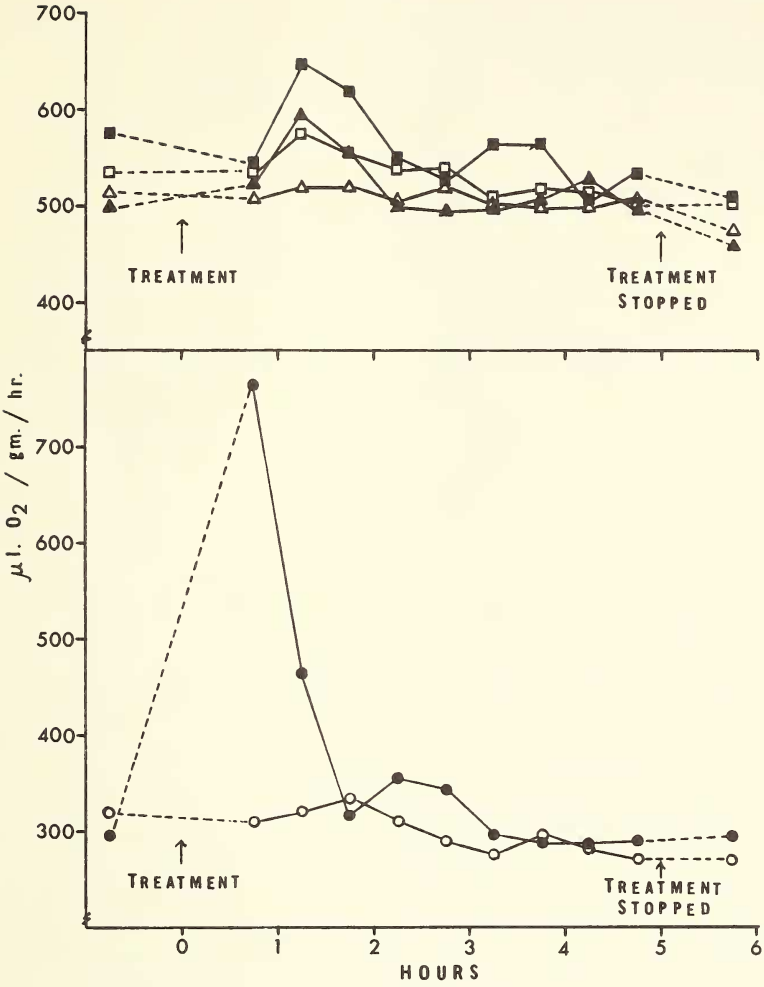

Fig. 2. Mean oxygen consumption by *Tenebrio molitor* before, during and after fumigation with carbon disulphide (0.12 gm/l.) for 5 hours. Upper: adults; lower: larvae. ■ 7 treated males, □ 2 control males; ▲ 7 treated females, △ 2 control females; ● 7 treated larvae, ○ 2 control larvae.

adults and larvae anaesthetized with chloroform whereas ethylene dichloride produced no significant effect (table 2). The legs of the adults treated with ethylene dichloride and carbon disulphide and those of control adults were folded, indicating tetanic contraction of the muscles.

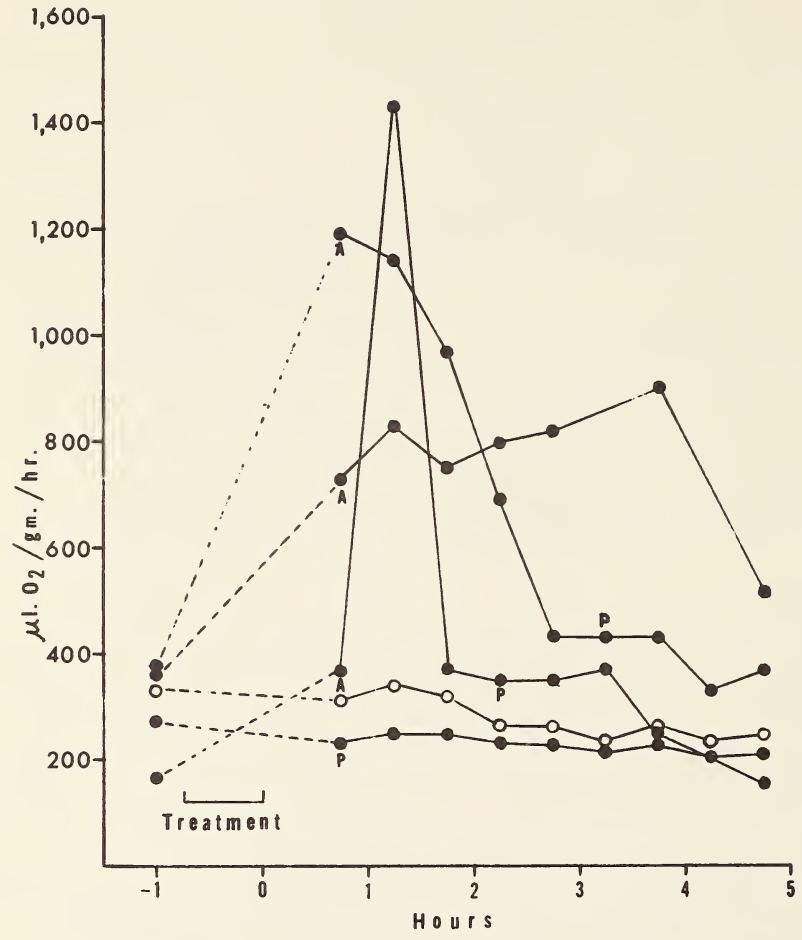

Fig. 3. Oxygen consumption by *Tenebrio molitor* larvae before and after treatment with ethylene dichloride (0.12 gm/l.) for 0.75 hour. ● treated; o control insect. A = active; P = paralysed.

Chloroform was found not to affect oxygen consumption of homogenized tissue of larvae after these were treated for 0.25 hr. The oxygen consumption by the control samples was 41 ± 6.1 µl O_2 gm/hr and that of treated samples was 38 ± 4.2 µl O_2 gm/hr as determined over a period of 2.5 hours.

Sorption of Fumigants by Insects

No further sorption of carbon disulphide by adult females occurred after 40 minutes or by larvae after 80 minutes (table 3). In each stage

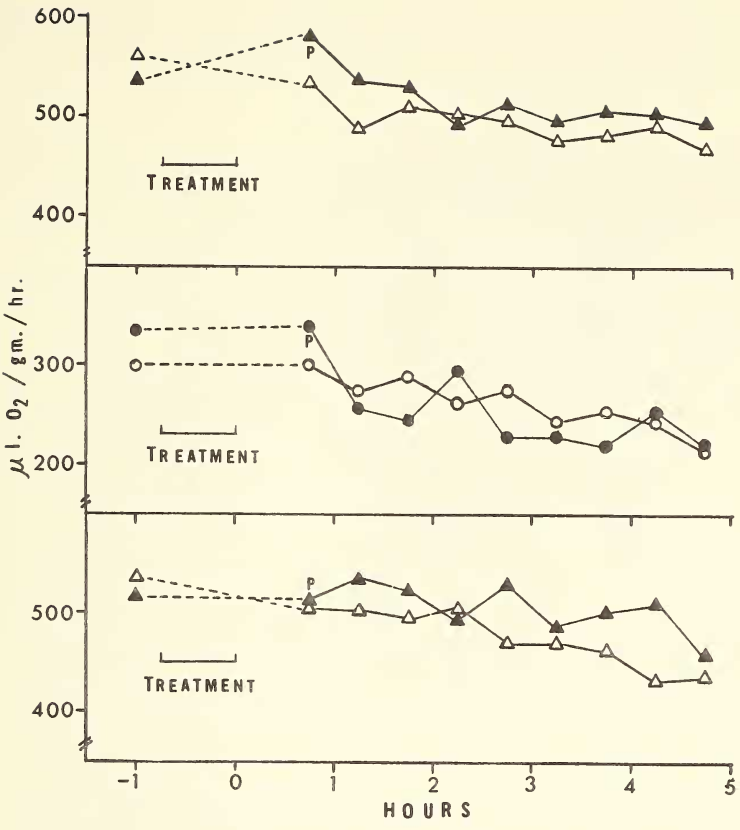

Fig. 4. Mean oxygen consumption by *Tenebrio molitor* before and after treatment. Upper: females, treated with ethylene dichloride (0.12 gm/l.) for 0.75 hr. Middle: larvae; Lower: females, both treated with carbon disulphide (0.12 gm/l.) for 0.75 hr. ▲ 7 treated females, △ 2 control females; ● 7 treated larvae, ○ 2 control larvae. P = paralysed.

most of the sorption took place within the first half of this period. Sorption of ethylene dichloride was much slower; there was no detectable change of weight of larvae in the first 20 minutes. Sorption of both materials was much quicker in adults than in larvae.

Effects on Abdominal Ventilation

In *T. molitor* adults, the normal ventilation mechanism is raising and lowering of the abdominal terga supplemented by protraction and retraction of the head and prothorax and longitudinal telescoping movement of the last few abdominal segments. When they were fixed to the molding clay, they struggled to free themselves and demonstrated the three types of ventilating mechanisms. For each test, two males and two females were used and the movements of the abdominal terga only were counted.

TABLE 1. Effects of fumigants on the oxygen consumption by homogenized tissue of adults and larvae of *Tenebrio molitor* 3 control and 4 treated samples in each test.

Test No.	Stage & sex	Fumigant	Dosages gm/lxhr	O_2 Consump. $\mu l\ O_2$/gm/hr	Period hrs.	t test value	Prob. of t
1	larv.	control		496.3 ± 15.9	6.5		
	larv.	EDC	0.12 x 1	429.4 ± 17.4	6.5	3.45	> 0.01
	larv.	CS_2	0.12 x 1	402.7 ± 9.9	6.5	5.29	< 0.01
2	larv.	control		472.7 ± 3.9	6.0		
	larv.	CS_2	0.18 x 2	351.6 ± 22.9	6.0	5.17	< 0.01
3	larv.	control		240.6 ± 8.0	5.5		
	larv.	EDC	0.18 x 2	132.1 ± 3.2	5.5	7.02	< 0.001
4	♀	control		461.2 ± 23.1	7.0		
	♀	EDC	0.12 x 1	449.3 ± 13.2	7.0	0.40	> 0.5
	♀	CS_2	0.12 x 1	341.3 ± 12.3	7.0	4.95	< 0.01
5	♂	control		385.4 ± 7.3	5.5		
	♂	EDC	0.18 x 2	305.1 ± 9.3	5.5	6.09	< 0.01
	♂	CS_2	0.18 x 2	214.5 ± 8.0	5.5	14.12	< 0.001
6	♀	control		562.6 ± 16.3	5.5		
	♀	CS_2	0.18 x 2	323.1 ± 7.5	5.5	17.40	< 0.001

EDC = ethylene dichloride

TABLE 2. Effects of fumigants on oxygen consumption by *Tenebrio molitor* anaesthetized with chloroform as determined over a period of 1 hour. 10 control and 10 treated insects in each test.

Test No.	Stage & sex	Fumigant	Dosages gm/lxhr	O_2 Consump. $\mu l\ O_2$/ gm	t test value	Prob. of t
1	larvae	control		316.1 ± 10.9		
	larvae	EDC	0.12 x 1	293.1 ± 12.8	1.37	> 0.1
	larvae	CS_2	0.12 x 1	238.3 ± 12.9	4.59	< 0.001
2	females	control		639.5 ± 19.2		
	females	EDC	0.12 x 1	601.9 ± 22.3	1.28	> 0.2
	females	CS_2	0.12 x 1	470.7 ± 13.5	7.21	< 0.001

EDC = ethylene dichloride

TABLE 3. Cumulative increase in weight of *Tenebrio molitor* due to sorption of fumigants during treatment.

Stage	Time (min.)	Total wt. gained mg.
CS_2		
females (10)*	20	12.7
	40	14.2
larvae (8)	20	8.0
	40	15.3
	80	20.8
EDC		
females (10)	20	2.3
	40	5.3
	70	11.5
	130	18.4
	190	24.4
	250	28.2
larvae (8)	20	nil
	40	1.6
	70	7.5
	130	15.1
	190	25.2
	250	36.0
	310	46.1

EDC = ethylene dichloride
* Figures in parentheses indicate the number of specimens.

Before ethylene dichloride was introduced into the chamber, the number of ventilation movements per minute was 18 ± 1 and after treatment, it increased to 26 ± 2 before they were paralysed when all movements stopped. When carbon disulphide was used, the abdominal tergal movements per minute before and after treatment were 19 ± 2 and 20 ± 2 respectively.

Time to Paralysis

Ten specimens of both larvae and adults were used to determine the length of time for paralysis to set in after treatment with ethylene dichloride and carbon disulphide. When the adults were on their backs with no movement of legs, and when the larvae showed only occasional very slow bending movements of the bodies, they were assumed to be paralysed. Table 4 shows that carbon disulphide paralysed larvae quicker than ethylene dichloride. Although both paralysed the adults faster than the larvae, the effect was more pronounced in ethylene dichloride. The time required for carbon disulphide to paralyse the larvae was less than twice that for paralysing the adults but the time required for ethylene

TABLE 4. Time in minutes to paralysis of *Tenebrio molitor* during exposure to fumigants.

Stage & sex	Fumigants	Time, mean (of 10) ± S.E.
0.12 gm/l		
larvae	carbon disulphide	18.0 ± 0.6
females	carbon disulphide	12.3 ± 0.3
males	carbon disulphide	13.1 ± 0.6
larvae	ethylene dichloride	65*
females	ethylene dichloride	12.0 ± 0.4
males	ethylene dichloride	13.3 ± 0.5
0.24 gm/l		
larvae	carbon disulphide	7.0 ± 0.5
females	carbon disulphide	4.6 ± 0.2
larvae	ethylene dichloride	35*
females	ethylene dichloride	4.5 ± 0.2

* To paralysis of all 10 specimens.

dichloride to paralyse the larvae was five times as long as for paralysing the adults.

Responses of the Spiracles of Adults to Fumigants

Observations were made only on the second and third pairs of spiracles because they are easy to see with the microscope. The second spiracle, located just posterior to the base of the elytron, is a trough-like structure dilated by a single muscle attached obliquely to a sclerotized bar along the anterior edge of the spiracular opening. The third spiracle, located on the first abdominal segment and posterior to the hind wing base, has a single valve-type closing apparatus (Snodgrass 1935). The anterior bar forms a bow and it is fixed and rigid. Opening and closing is effected by the movement of the posterior bar which has an L-shaped sclerotized lever with both dilator and occlusor muscles attached to it.

When ethylene dichloride was introduced to a chamber containing a resting beetle with the third spiracles closed the second spiracles stayed open all the time whereas the third spiracles opened but closed when fresh air was readmitted. Thus the action of ethylene dichloride is reversible. Ethylene dichloride always caused the second and third spiracles to open. Whenever the spiracles opened, they remained open until the beetle was exposed to fresh air. Tests were also done with adult *Blattella germanica* (L.). Both first and second spiracles possess an occlusor muscle and opening is effected by the elastic nature of the spiracular closing, the roaches showed much excitation and there was rapid fluttering

of both spiracles.

Only the third spiracle was used in isolation because it remained closed after isolated. Variable results were obtained. The responses were slow, for example one spiracle only started to open 5 minutes after ethylene dichloride was introduced and 25 minutes later it was only open half-way.

In order to see how the spiracles would respond to ethylene dichloride when only the central nervous system was treated, the ventral nerve cord was exposed by dorsal approach and ethylene dichloride was applied to the anterior end of the ventral nerve cord in the prothorax with a very small fumigant soaked cotton wad. In all tests, both the second and third spiracles opened in response. To see if application of ethylene dichloride to the peripheral nerves would result in similar changes, the tarsus of an insect was brought into contact with a small cotton wad moistened with fumigant, but this failed to cause the spiracles to open. Instead, both second and third spiracles showed repetitive fluttering.

When the beetles were treated with carbon disulphide, the second spiracles always opened but no consistent results were obtained with the third spiracles. The responses of the spiracles in one individual after carbon disulphide was introduced were studied in greater detail. The second spiracle, once it had responded to the fumigant by opening, remained open even after it was exposed to fresh air. The third spiracle closed after carbon disulphide was introduced into the chamber but opened after exposure to fresh air. It closed again when carbon disulphide was readmitted. This reversibility as found in ethylene dichloride shows the narcotic nature of the two compounds. When *Blattella germanica* were similarly treated, in all four tests the first two spiracles closed when paralysis took place. No consistent results were obtained when carbon disulphide was applied to isolated spiracles.

When carbon disulphide was applied locally to the ventral nerve cord, both the second and third spiracles opened as long as the cotton wad remained in contact, but the third spiracle resumed normal movements shortly after the cotton wad was removed. Application of carbon disulphide to the tarsus only caused some excitation of the beetles and more rapid movements of the spiracles.

Effects on the Ventral Nerve Cord and Muscle

To obtain further evidence of the effects of carbon disulphide and ethylene dichloride on the electrical activity of the ventral nerve cord, male *Periplaneta americana* were used. The ventral nerve cord of roaches which had been paralysed by ethylene dichloride or carbon disulphide, did not show the spontaneous activities detected in the control specimens. Spontaneous activity began to appear shortly before the roaches started to stir. But local application of these fumigants to the ventral nerve cord with a micro-syringe produced repetitive discharges.

In the studies on spiracular movements, muscle contractions are involved. Further evidence was obtained by perfusing one microlitre of either fumigant into an isolated leg of *Periplaneta americana* with a micro-syringe. The coxa and femur were flexed together and they remained

in that state for 1.5 hours when observation stopped. The same was found with femora and tibiae of isolated legs of *Tenebrio molitor* adults when the cut ends of the legs were brought into contact with a small cotton wad soaked with the fumigant.

When isolated legs of adult *Tenebrio molitor* were put in a petri dish and ethylene dichloride was injected into the dish, the femora and tibiae were flexed together in 35 seconds after ethylene dichloride was introduced. On exposure to fresh air, they gradually extended. The same results were obtained with isolated legs treated with carbon disulphide except that flexing occurred in 25 seconds.

It was noted before that after 5 hours of fumigation with ethylene dichloride, the bodies of many larvae of *T. molitor* became flaccid, some died within 5 days, but some survived beyond that period. In the latter, ethylene dichloride seemed to cause some permanent injury either to the nerves or to the muscles for many of them never regained their locomotive power and one of them molted to the next instar the second day after treatment but was unable to shed the old cuticle.

DISCUSSION

Carbon disulphide had no effect on oxygen consumption by *Tenebrio molitor* adults either during or after treatment. Although there was an increase of oxygen consumption by *Tenebrio molitor* larvae during treatment with carbon disulphide, this increase did not persist after the treatment was ended. It is suggested that there was only sorption of carbon disulphide by the insect tissues during the treatment. This is confirmed by the increase of weight of insects during treatment and agrees with the observations of Shafer (1911) and McKee *et al.* (1943) that insects and vertebrate tissues can become saturated with carbon disulphide. Shafer (1911, 1915) found that carbon disulphide inhibited oxygen consumption by living *Passalus cornutus* but reanalysis of his data in the earlier paper shows that there was no significant difference between the control and the treated specimens and also that there was no significant difference in respiratory quotients between them. In his later paper Shafer (1915) used low, high, and nearly saturated concentrations. When a low concentration was used, in 29 hours, the oxygen consumed was 5.5cc by the two treated insects and it was 6.4 cc by the two control insects. It is hard to say whether the difference is significant. In high and nearly saturated concentrations, the controls used 6 - 16 times more oxygen than the treatments. With these high concentrations, the insects probably only survived a few hours and not as long as the period during which the oxygen consumption was measured (16 - 24.5 hours). My data agree reasonably well with Shafer's (1911) data in that there was no significant difference between the oxygen consumption by the control and by the treated insects when active insects were treated with lower doses. However, I found that when insects were first anaesthetized with chloroform, the control specimens consumed more oxygen than the treated. That the unanaesthetized carbon disulphide treated insects did not show a decrease in oxygen consumption was probably because this was offset by an increase

in oxygen consumption because of muscular contractions as exemplified by the folding of the legs. Since the legs of the adults anaesthetized with chloroform were also folded indicating tetanic muscular contraction, the muscles of the treated and the control insects were in the same state and only then the intrinsic effect of carbon disulphide could be seen. The data obtained with homogenized tissues confirm the data obtained with anaesthetized insects and also confirm the observations of Shafer (1915) using tissue extract of *Passalus cornutus*. I found that the extent of decrease of oxygen consumption in the treated samples depends on carbon disulphide concentration, inhibition being greater in adults than in larvae. The present data do not agree with those obtained with rabbit tissue by De Meio and Brieger (1949).

No previous work has been done on the effect of ethylene dichloride on respiration. The present work with active insects indicates very clearly that ethylene dichloride causes an increase in oxygen consumption and that there is some correlation between oxygen consumption and the activities of the treated insects. Ethylene dichloride did not increase tissue respiration but rather the effect is through the action on activities. Studies with homogenized tissue showed that a lower dose of ethylene dichloride did not affect oxygen consumption significantly, but a higher dosage did decrease oxygen consumption. The cause of inhibition of oxygen consumption by higher doses of ethylene dichloride is not known but these results correspond well with the finding (Winteringham & Hellyer 1954) that longer exposure of *Musca domestica* caused considerable depletion of ATP and arginine phosphate. Insects paralysed by ethylene dichloride showed no decrease in oxygen consumption although it is expected that cessation of activities would be accompanied by such a change; probably contraction of muscles caused by these fumigants after paralysis accounts for this maintained rate of oxygen consumption. No difference was found in oxygen consumption of the anaesthetized treated and control specimens, the reason being that the muscles of the treated and control were in about the same state of contraction.

Ethylene dichloride resembles many contact insecticides such as chlorinated hydrocarbons, dinitro compounds, nicotine, pyrethrin, and organic phosphates which cause an initial increase of respiration and then a decrease towards the normal level, and these correlate with initial excitation and eventual paralysis of the treated insects (Harvey & Brown 1951).

In pyrethrins poisoning, the initial excitatory phase has been attributed to the stimulation of the peripheral sensory nerves (Hutzel 1942 a, b, Page *et al.* 1949). The larvae of *Tenebrio molitor* treated with ethylene dichloride developed symptoms similar to those of caterpillars poisoned by a median lethal dosage of pyrethrins (Brown 1951). The convulsions which succeed the initial excitatory phase in pyrethrin poisoning are attributed to stimulation of the central nervous system and the progressive paralysis is attributed to the onset of pathological changes in the nervous system (Brown 1951). Lowenstein (1942) found that under the influence of pyrethrins, the initial excitatory phase was marked by a massive discharge of a number of impulses and that a spontaneous synchronized discharge of continuous trains of giant-fibre potentials became

prominent. It is possible that ethylene dichloride acted essentially in the same way as pyrethrins. There is evidence that ethylene dichloride caused initial excitation of the ventral nerve cord, since the insects were always in a state of excitation after the application of this chemical and prior to paralysis. Although it has been shown that local application of ethylene dichloride and carbon disulphide to the ventral nerve cord caused an increase in spontaneous nervous activity, this could be due to the contact action of the cotton wool on the ventral nerve cord.

McGovran's (1932) finding that carbon disulphide increased the average rate of tracheal ventilation of the grasshopper *Arphia sulfurae* (Fab.) in the first five minutes may be disputed because of his technique. He confined the thorax in a small chamber containing carbon disulphide with abdomen in a separate chamber containing air. The decrease in pressure of the chamber containing the thorax was taken as the amount of air ventilated from the first chamber through the thorax and abdomen to the second chamber, preventing him from distinguishing between sorption of carbon disulphide and ventilation. Insect and vertebrate tissues quickly become saturated with carbon disulphide as has been shown here and previously by McKee *et al.* (1943) and Shafer (1911). Ethylene dichloride certainly increased the rate of ventilation before the insects were paralysed since hyperactivity demands more oxygen and the wriggling movements of the larvae are likely to increase ventilation. It was demonstrated here that ethylene dichloride, but not carbon disulphide, caused an increase in abdominal ventilation movements in *T. molitor* adults before they were paralysed.

Carbon disulphide acts faster than ethylene dichloride. This can be attributed to the fact that carbon disulphide vapour has a greater penetrating power (Sun 1947) since it has a lower molecular weight and it may depress or abolish the peripheral nerve potential and conductivity as found in Japanese toad *Bufo vulgaris japonicus* (Echikawa 1959a). The fact that ethylene dichloride was as quick as carbon disulphide in paralysing adults may be due to its effect on the spiracles, since both second and third spiracles were completely opened during fumigation with ethylene dichloride. In the larvae, however, all spiracles were closed and they showed no visible movements.

When the anterior part of the ventral nerve cord was treated with either fumigant, the spiracles always opened. However, when carbon disulphide was applied in vapour form to the whole insects, variable results were obtained. It seems unlikely that the response of the spiracle depends on the local concentration of carbon dioxide, for when isolated spiracles are exposed to carbon disulphide vapour, carbon dioxide can easily diffuse out.

Echikawa (1959b) found that the skeletal muscle fibres of the toad were non-reactive to carbon disulphide, but the motor end-plates showed spontaneous activity after treatment. When skeletal muscle was induced to contract by carbon disulphide, it would remain in such a state for a long time, although fatigue of the motor end-plate occurred readily in the presence of carbon disulphide. External force was required to stretch the muscle. A similar phenomenon was observed with muscles of isolated legs of both *Periplaneta americana* and *Tenebrio molitor* when they were perfused

with, or treated with the vapour of, either carbon disulphide or ethylene dichloride. These fumigants probably act on motor end-plates in insect muscles as in the toad. If carbon disulphide and ethylene dichloride can really act on the motor end-plates in spiracular muscles they can cause both the dilator muscle and occlusor muscle to contract, and it seems that it is the net result of these forces that cause the third spiracle to open or to close. Which of the two muscles exerts a greater force might depend on physiological conditions.

When whole insects were treated with ethylene dichloride vapour, the third spiracle always opened. The difference of responses given by intact and isolated spiracles seems to suggest that the ventral nerve cord was responsible for the consistent opening of the third spiracle in the presence of ethylene dichloride vapour. Miller (1960b) found that in the desert locust, *Schistocerca gregaria* (Forskal), some spiracles have antagonistic muscles as in the third spiracle of *Tenebrio molitor* and two different types of action potentials are involved for their movement. Usually, only one type of action potential could be recorded from the transverse nerve and only one of the muscles is functioning all the time. In *Tenebrio molitor* judging from the structure of the third spiracle, opening of the spiracle needs active contraction of the dilator muscle and relaxation of this muscle would bring the valve back to the closed position. The occlusor muscle is probably only used for active closing. It is suggested that when the ventral nerve cord produced massive discharges in ethylene dichloride only one type of action potential was produced and so tetanic contraction only occurred in the dilator muscle.

These results point to the importance of spiracular structure. For example, if all the spiracles have only dilator muscles, then during fumigation they would all open and facilitate the entrance of the fumigant. Insects with only occlusor muscles to the spiracles, would have these all closed during fumigation. This importance of spiracular structure has been pointed out by Sharplin & Bhambhani (1963) in their study of spiracular structure and water loss under reduced pressure. The responses of the spiracles depend on their structure and the nature of the fumigants, having no essential relation to the effects of fumigants on respiration.

Carbon disulphide and ethylene dichloride are narcotics but narcosis is not the cause of death of insects (Brown 1951). Hurst (1945) thought that narcosis may involve the indirect blocking of enzyme activity by the adsorption of insecticides on the protective lipo-protein components of the nervous tissue. The narcotics are known to inhibit respiratory enzymes (Shafer 1911, 1915, McKee *et al.* 1943, Anonymous 1949, Baldwin 1952) but ethylene dichloride has not been shown to have such an effect. Fukami *et al.* (1959) showed that there is a positive correlation between the action of rotenone on nerve conduction and inhibition of respiratory metabolism; the rotenone derivatives which have a potent inhibitory action on metabolism block nerve conduction. It seems very likely that the cause of inhibition of nerve conduction in peripheral nerves of the toad by carbon disulphide (Echikawa 1959a) is the inhibition of the succinic oxidase enzyme system which is important in normal nerve tissue metabolism (McKee *et al.* 1943). If this is true, then carbon disulphide might

also interfere with insect peripheral nerve conduction.

In conclusion, ethylene dichloride increased the oxygen consumption by larvae but not adults; it had a very slight effect on larval tissue homogenate but only very high doses had an effect on the oxygen consumption by adult tissue homogenate. Carbon disulphide had no effect on oxygen consumption by normal insects but the oxygen consumption by anaesthetized insects was depressed. Tissue homogenate was affected by carbon disulphide, the extent of depression of oxygen consumption depending on the dosages applied. Although both ethylene dichloride and carbon disulphide were taken up by insect tissue during fumigation, sorption occurred more quickly in carbon disulphide, due to the lower molecular weight and hence higher penetration speed. The increase in abdominal ventilation movements caused by ethylene dichloride, is probably due to its stimulating effect, causing hyperactivity of the insects.

The larvae were paralysed by carbon disulphide much more quickly than by ethylene dichloride and this again is related to the difference in molecular weight and hence penetrating speed of these two fumigants. In adults, the greater molecular weight of ethylene dichloride was compensated for by its effect on ventilation and the spiracles, so that both of these fumigants paralysed the adults in about the same time.

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CORRIGENDA

P. 224 (vol. II, no. 3). For *Chematopsyche analis* (Banks), read *Cheumatopsyche analis* (Banks); for *Trianodes marginata* Sibley, read *Triaenodes marginata* Sibley; for *Leptocalla exquisita* (Walker), read *Leptocella exquisita* (Walker); for *Agapetus hessi* Leonard & Leonard, under ♂, for 0 read 1.